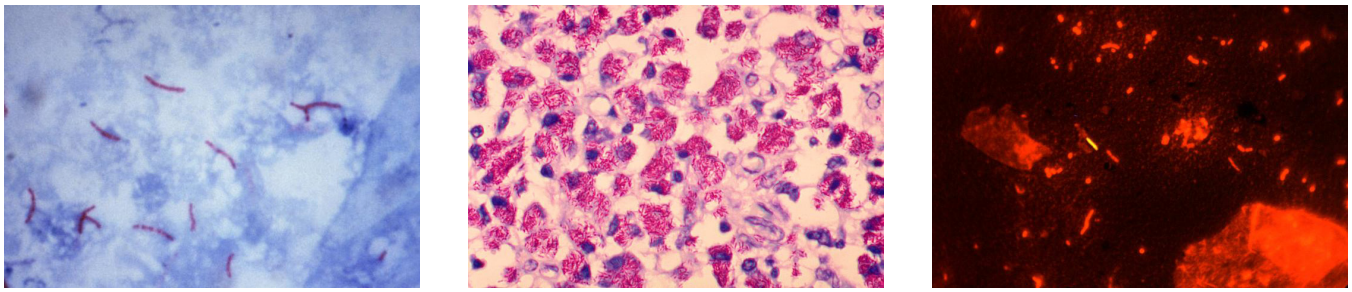
Mycobacteria

Introduction

Mycobacteria are **acid-fast rods**. All *Mycobacterium* species are **obligate aerobes**—they not only prefer to be in oxygen-rich tissues, but they absolutely cannot tolerate anaerobic conditions. All *Mycobacterium* species are **facultative intracellular** organisms, surviving in tissue and within the macrophages that try to kill them. And this is the thing with mycobacteria: they don't actually DO anything other than survive really well; all the damage they cause is due to the host's immune response trying to kill them off. These organisms **grow slowly** and **survive**. Because of this, the host immune system has to find a way to keep them at bay. They survive phagocytosis (macrophages don't work) and can be intracellular organisms (antibodies don't work), so what the body does is wall them away inside **granulomas**.



(a) (b) (c)

Figure 14.1: Mycobacteria
Several techniques allow identification of acid-fast organisms that will not stain with a Gram stain, aka mycobacteria. (a) A normal acid-fast stain has a blue background with scattered red elongated organisms. (b) A warm acid-fast stain has a yellow background, red rods, and some blue nuclei of host immune cells. (c) Immunofluorescence of acid-fast organisms is the auramine-rhodamine stain—if the slide lights up, mycobacteria are present; if it doesn't, there are no mycobacteria. Note that all these stains merely denote the presence of an organism that will stain acid-fast, not that it is mycobacteria or which species of mycobacteria it is.

The thing that makes them **acid-fast** is the consistency of their cell wall. The cell wall also provides them with resistance to detergents, common antibacterial antibiotics, and many disinfection procedures. Mycobacteria are unique bacteria in that their cell wall is very unlike any other bacterial cell wall. There is a small layer of peptidoglycan cell wall, just like any other bacteria. But on top of that is a layer of arabinogalactan (just recognize this word as one-of-the-things-in-mycobacterial-cell-wall). On top of that is another layer of lipids, long-chain lipids called **mycolic acids**. Mycobacteria get their name from the mycolic acid. The **high concentration of lipids** prevents the uptake of Gram stain, and the lipids are the target for **acid-fast stains**. Since only mycobacteria have mycolic acid, they should be the only organism to stain for mycolic acid (*Nocardia* also does a little, but all other bacteria do not). In addition to varying acid-fast preparations, there is an immunofluorescence technique, the auramine-rhodamine stain, which lights up only when there are mycobacteria (and nocardiae). The mycolic acid layer also prevents our typical antibiotics from getting to the plasma membrane. Mycobacteria are hard to kill and are acid-fast, recalled by the phrase, *"acid-fast is steadfast."*

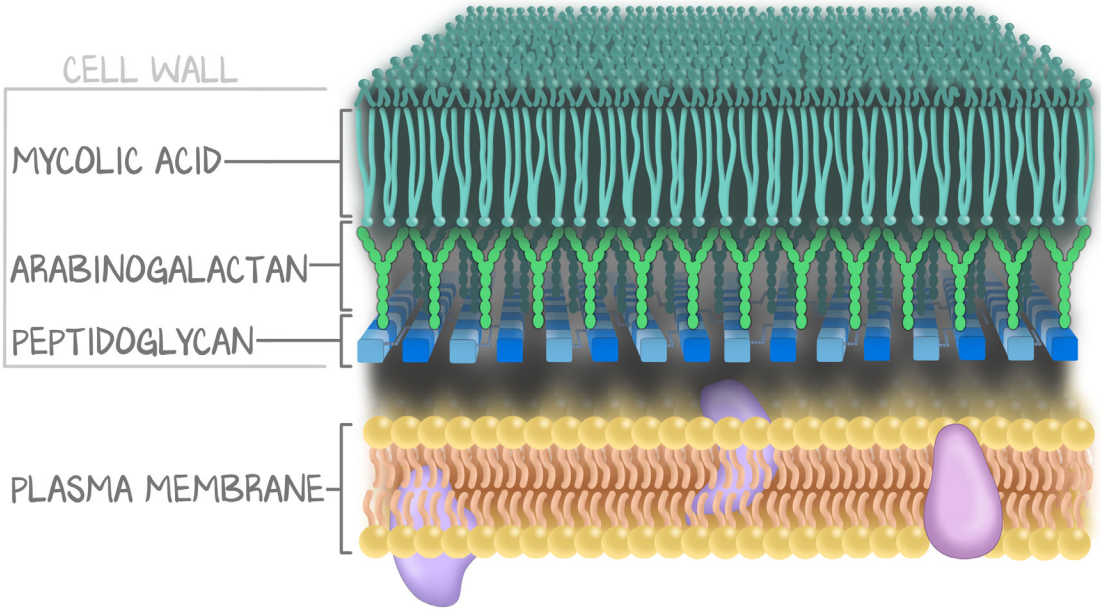


Figure 14.2: Mycobacterial Cell Wall
Unlike most bacteria that use a peptidoglycan cell wall, mycobacteria use a complex multilayered cell wall. There is a small peptidoglycan cell wall, such that, if the rest of the cell wall is removed by prolonged Gram staining, these organisms could stain weakly Gram negative. The majority of their cell wall consists of arabinogalactan and mycolic acid. The most you need to know about them is that because they are unique to mycobacteria, those structures are the target for treatment of mycobacterial species.

Of all these organisms, *Mycobacterium tuberculosis* (TB) is by far the most important to learn. Second is *Mycobacterium leprae*, only because of its history and obvious polarity in its two presentations. *Mycobacterium avium* complex (MAC, which is now the same thing as *avium-intracellulare*, MAI) is relevant for AIDS patients, but usually only in the form of prophylaxis against it. The others are included for thoroughness, but need not be considered unless score augmentation is the goal. We spend a long time on TB's pathogenesis and diagnosis in this lesson. We also have an antibacterial lesson dedicated to *Mycobacterium* which again focuses primarily on TB.

Mycobacterium tuberculosis ("Tuberculosis" or "TB")

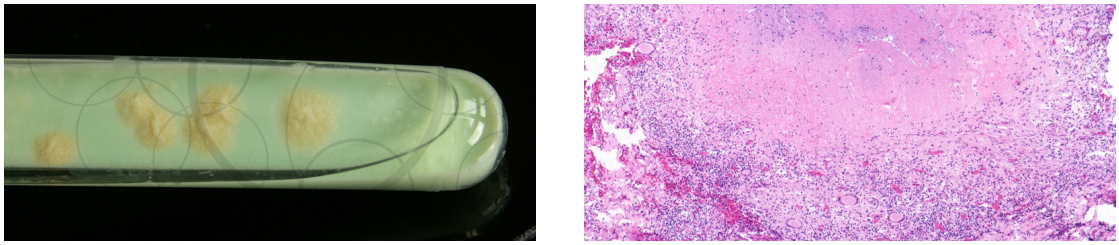
TB is by far the highest-yield organism. There is a low incidence of TB in the United States, under 10,000 new cases per year. Almost all cases are either in reactivation because of immunocompromise (AIDS, chemotherapy, and biologic rheumatologic drugs) or in the marginalized—homeless and incarcerated. Worldwide, there are close to 10 million cases per year, most of them in heavily populated developing countries—Brazil, India, China, Africa.

Physiology/Structure. TB is a *Mycobacterium* species, so is an obligate aerobe that stains **acid-fast**. It will turn fluorescent apple green on an **auramine-rhodamine stain**. It is a bacillus, rod-like, and can stain weakly Gram positive.

Virulence = survival. TB's "virulence" is actually more like "survival." It does not even induce disease—the immune response to TB does. TB is inhaled in droplets from another infected individual. The bacteria are distributed throughout the lung. Alveolar macrophages identify TB, and phagocytose the bacteria. TB is a **facultative intracellular** bacterium, which means it can live inside other cells. TB **inhibits phagosome-lysosome fusion**. This permits TB to remain alive within phagosomes. It also allows for the phagosome to become the TB transporter, moving about the cell, fusing with other

organelles, extracting nutrients. Even if fusion occurs, TB has a **heat-sensitive catalase**. It is a catalase, so it resists the hydrogen peroxide made by the initial respiratory burst (like *Staph. aureus*). But it is heat sensitive, so at the temperature the catalase test is done in the lab, it does not react, but at the temperature inside a human, it would. TB does not have any toxins, cytolysins, or any other such thing. It just lives.

Virulence 2 = granulomas. Phagocytosis by alveolar macrophages traps TB in a phagosome. The phagosome does not fuse with the lysosome. TB grows. The macrophage knows. The macrophage sends out IL-12 and TNF-α. Feeling the intracellular parasite, the adaptive immune system responds. T cells differentiate into Th1 helper T cells, feeding IFN-γ to infected macrophages, further activating them. Within the cell, macrophages make more lysosomes, fuse more lysosomes, and produce more nitric oxide—do more of the things they know how to do, tipping in favor of bacterial killing. Macrophages become **epithelioid cells**, their cytoplasm abundant from all the lysosomes, so that they appear not to be a macrophage, but instead an epithelial cell. Macrophages get so activated that the epithelioid cells eventually fuse to form **giant cells**. This mechanism attempts to contain the TB within a cell. Recognizing that the macrophages are failing, the immune system does something extra. It tells the giant cells to sacrifice themselves, forming the central core of necrotic mass of the **necrotizing granuloma.** TB is now tucked away in its prison, surrounded by the granuloma, the granuloma watched over by macrophages, CD4, and CD8 cells.



(a) (b)

Figure 14.3: Tuberculosis Diagnosis
(a) A Lowenstein-Jensen agar grows tuberculosis over a period of 6 weeks. (b) If biopsied, the hallmark of tuberculosis is caseating granulomas. Caseous means necrotic, so there is pink material (the granuloma) without any purple dots in it (no cells, because caseous). Surrounding the pink wasteland that is the granuloma, there are many purple nuclei lining it, the leukocytes that have built the defensive perimeter containing the tuberculosis.

If the bacteria burden was small, the TB is eradicated. If the bacteria burden was large, the granuloma is walled off by fibrin, preventing the immune cells from getting to any remaining TB. The leftover TB lies in wait, dormant for years or decades, until **reactivation** occurs, often a result of impaired immunity or old age.

Epidemiology. TB is spread person-to-person; **humans are the only natural reservoir**. The greatest risk factor is being near other people who have TB. Places where people are kept quite close to one another (**jails**, **homeless shelters**) and the people who provide for those people kept quite close to one another (**health care providers**) have the greatest risk. Outside the US the problem is much greater. The more densely packed a city, the easier it is for TB to spread. Poor, large cities lack the health care to manage the disease and provide the masses to infect. Because TB grows so slowly, even in a human host, a patient may be infectious and not know they are infected.

Diseases. Within the United States, TB is a pulmonary disease. Miliary TB, disseminated TB, and TB in any other organ is possible. They are not seen in the US. You should be aware of miliary TB but be extensively knowledgeable in primary TB—a pneumonia—and reactivation TB—a cavitary lung disease presenting with hemoptysis, fever, and night sweats.

Primary pulmonary TB (first exposure). TB acts, to a naïve alveolar macrophage, much like any bacterium. The initial presentation resembles pneumonia with a fever and a cough. The patient is infected and is infectious. Macrophages phagocytose the TB, but TB evades lysozymes, then replicates within macrophages. These macrophages die. The ones that survive long enough bring their antigen to regional lymph nodes and signal their distress. The regional lymph nodes become **Ghon complexes**, usually seen as mediastinal lymphadenopathy. The immune system walls off the TB. Organisms walled off within the Ghon complex remain viable. This is where the granulomas form. Granulomas are microscopic lesions, seen only on histology. Cavitary lesions are not seen in first exposure, and the X-ray may be normal. Primary TB, like most pneumonias, affects the middle and lower lobes.

Reactivation TB. This is what most people think of when they hear "TB." Erosion of the granuloma, loss of the defensive perimeter secondary to reduced T-cell immunity (age, time, AIDS, etc.), allow the dormant TB to awaken and initiate replication. The patient presents with **fever**, **night sweats**, and **hemoptysis**. The hemoptysis is an attempt to contain the TB, killing healthy lung tissue along with the infected cells. Because TB is an **obligate aerobe** (it really likes oxygen), the reactivation tends to occur where the oxygen tension is highest—**the upper lobes** and **apices**. Diagnosis is made with imaging, presenting on CT scan with a tree-in-bud pattern, or may result in **cavitary lesions** seen on X-ray. In primary TB, the infection was established wherever the TB landed in the lung. On reactivation, upon escaping from its prison, TB goes to where it grows best.

Miliary TB. When TB leaves the pulmonary system and disseminates, it is called miliary TB. When in the spine, it is called Pott's disease. Miliary TB can go to the GI, skin, kidneys, lymph nodes, and liver.

Diagnosis. Diagnosis comes down to screening (PPD or interferon-gamma release assay [IGRA]), lung assessment (Chest X-ray), active disease (AFB smear), and confirmation of TB (culture).

While it may be academically true that the presence of a BCG vaccine may cause a false-positive PPD and may cause even cause a false-positive γ-interferon assay (the newer IGRA is not affected), the texts that state this fail to recognize clear practice guidelines. The IDSA and the CDC both state that interpretation of TB screening tools should be agnostic of BCG vaccination. You treat a person vaccinated with BCG exactly the same as someone who has not received a BCG vaccination. While the IGRA is preferred for screening patients with a BCG vaccination, "a *person with a history of BCG vaccination can be tested and treated for [latent TB infection] if they react to the TST [tubercular skin test]. TST reactions should be interpreted based on risk stratification regardless of BCG vaccination history*."[1] The **BCG vaccine** is not used in the United States. It protects against miliary TB. When a person has had the BCG vaccine, it does **not alter the screening process**.

1. https://www.cdc.gov/tb/publications/ltbi/pdf/targetedltbi.pdf, p. 12

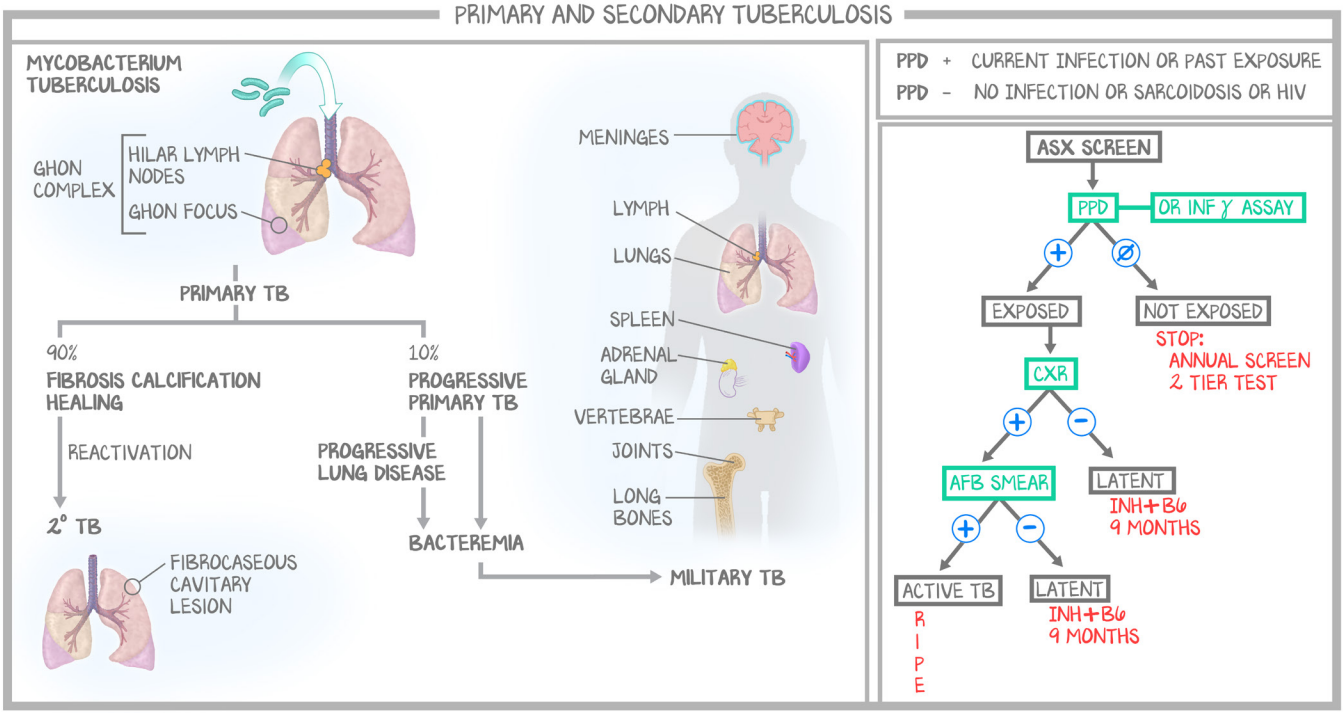


Figure 14.4: Tuberculosis
On the left is the vastly complicated milieu of tubercular disease. The vast majority in the United States is primary pulmonary TB, which targets the lower and middle lobes, presents as pneumonia, and has Ghon complexes (bacteria in the lung with lymphadenopathy). The tuberculosis that health care providers think of is secondary TB, reactivation of pulmonary TB, resulting in cavitary lesions, hemoptysis, and weight loss. The most dangerous form of TB, found primarily outside the United States, is miliary TB, where TB spreads throughout the body. TB is the number one cause of adrenal insufficiency outside the United States. The BCG vaccine is to prevent miliary TB in children in developing countries. On the right is the screening process for an asymptomatic patient (aka you every year for the rest of your life). The screen begins with either a PPD or IGRA (equivalent, except if BCG vaccine has been received). If positive, a chest X-ray is obtained. If positive, AFB smears are the confirmatory test. The nomenclature and treatment protocols are more clinical, though visualizing—PPD, then CXR, then AFB—is crucial for your training now.

Active TB is diagnosed by smear. Gram stain with microscopy (weakly Gram positive) and auramine-rhodamine (apple green) are fast, but they are only clues. The definitive diagnosis is made with **acid-fast stain** and eventual **acid-fast culture** (3–6 weeks on Löwenstein-Jensen agar). There are different types of acid-fast staining (truant fluorochrome, Ziehl-Neelsen, Kinyoun) but to you, they are all "acid-fast," and you need not separate them.

Screening for TB can be performed either with the **PPD** tuberculin skin test or the **interferon-γ release assay**. Screening assesses only if someone has been exposed. A patient who had primary TB will have a reaction to the antigen. A patient who has never been infected will have negative tests. The IGRA is a **one-time test** that says yes or no. The PPD is placed under the skin and must be read in 48–72 hours. The measurement is made on the **zone of induration**. For **high-risk patients** (close contacts, HIV, transplant) the cutoff is > 5 mm; for **moderate-risk patients** (health care worker, inmate, homeless, regular exposure) the cutoff is > 10 mm; and for **low-risk patients** (soccer moms, non-health care people) the cutoff is > 15 mm.

If ever the patient has a positive PPD or IGRA, they should never again receive the test, as it will always be positive. Continued screening is performed with an **X-ray**. The first time someone tests positive for a screening test, they get an X-ray as well. If there is no active disease, they are said to have **latent TB**. If they have evidence of active disease on X-ray, a **sputum** is done as above. If they are sputum negative, they are latent TB.

Treatment and Prevention. If a patient tests positive for the screen, and negative for active infection, they are said to be **latent TB**. Latent TB is treated with **isoniazid for 9 months**; don't forget your B_6.

If a patient tests positive for the screen and has a positive AFB smear, they are **active TB**, and are treated with **RIPE**: **R**ifampin, **I**soniazid, **P**yrazinamide, **E**thambutol. We discuss these drugs in detail in Antibacterials #6: *Antimycobacterial*.

Mycobacterium leprae ("Leprosy")

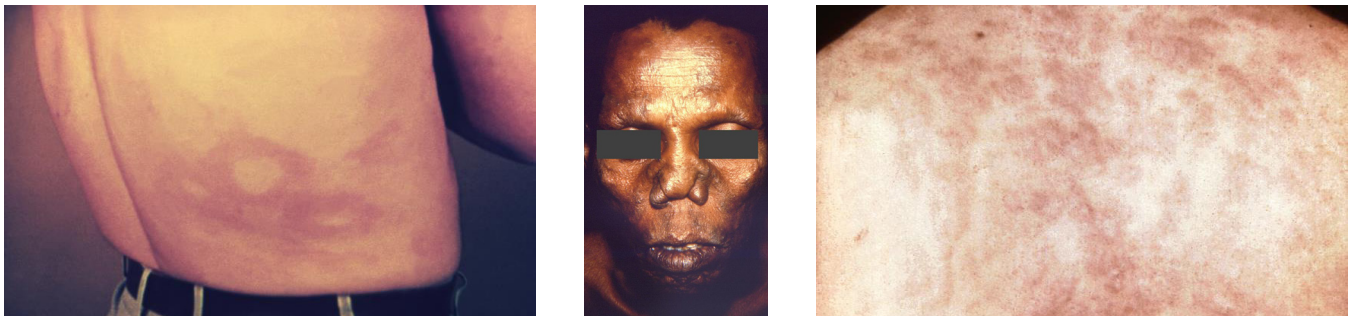
Leprosy is a skin disease that is actually two distinct diseases, both caused by the same organism. *Mycobacterium leprae* is an **obligate intracellular** organism that can be transmitted only from human to human with prolonged exposure, and some armadillos in Texas and Louisiana have it, too. While those armadillos may represent an endemic source in the United States, humans are believed to be the **only true reservoir**. Fewer than 100 cases in the past 7 years have been reported in the United States, almost all of them immigrants from the Pacific islands or Mexico. It is the **slowest growing bacteria**, with a doubling time of 2 weeks. It is treated with **dapsone** and **rifampin**, and treatment lasts for 2 years. **Isolation is not required**—there is no need for a "leper colony." The **lepromin test** is the analog to the PPD of TB. The lepromin test can only be positive in the tuberculoid leprosy variant. Two diseases—tuberculoid leprosy and lepromatous leprosy—from one bug—*Mycobacterium leprae*.

Tuberculoid leprosy is the result of a **strong cell-mediated response**. Because of the strong immune response, mitigated by Th1 cells, the organisms are contained, and there are few organisms in the skin. It is called tuberculoid leprosy because Th1-macrophage-induced granuloma formation is the pathogenesis of tuberculosis, and the pathogenesis of tuberculoid leprosy. In this variant, the *leprae* live and are contained in granulomas of the nerves. This causes **patches** of skin that are **well demarcated,** raised, and have **complete sensory loss.** Visible enlargement of the nerves occurs as the granulomas swell. These lesions are macular and few in number. Being well contained, the **infectivity is low**. With a robust immune response, there will be a **positive lepromin test.** Because the granulomas contain the organisms, the adaptive immune system does not see those organisms, so immunoglobulin levels return to normal.

FEATURES	TUBERCULOID LEPROSY	LEPROMATOUS LEPROSY
Skin lesions	Few lesions, well circumscribed, hypopigmented, total anesthesia	Diffuse, poorly circumscribed macules, papules, or nodules; extensive tissue destruction (e.g., nasal cartilage, bones, ears); diffuse nerve involvement with patchy sensory loss
Nerve	Nerve enlargement	No nerve enlargement
Histopathology	Granulomas, few acid-fast bacilli, Langerhans cells present	Predominantly "foamy" macrophages with few lymphocytes; lack of Langerhans cells; numerous acid-fast rods in skin lesions and internal organs
Infectivity	Low	High
Immune response	Strong cell-mediated immunity, Th1 response	Weak cell-mediated immunity, Th2 response
Lepromin	Very reactive	Not reactive
Immunoglobulins	Normal	Elevated

Table 14.1: Leprosy Types

Lepromatous leprosy is what a weak immune system does in response to *leprae*. This is a Th2 response and, more importantly, not a Th1 response. It represents a failure of macrophages to contain the bacteria, and so, with a weakened immune system, results in dissemination. Without a robust immune response, the lepromin skin test is negative. There will be **nodular skin lesions** with **multiple organs involved**. This causes **leonine** (lion-like) facial changes. Numerous discrete lesions coalesce to form nodules. The face is deformed with loss of eyebrows and the nasal septum. Because the organisms are not contained to the nerves, there is only **patchy sensory loss** and the absence of nerve enlargement. The immune system has lost control over the bacteria and is trying to get it back. **Immunoglobulins are elevated** but are failing. When punch biopsy is done, there are few granulomas and many organisms. These patients **are infectious**.



(a) (b) (c)

Figure 14.5: Leprosy
(a) Tubercular leprosy is well demarcated, hypopigmented, and few in number. (b) Leonine facies of lepromatous leprosy. (c) Lepromatous leprosy has multiple poorly demarcated skin lesions.

Mycobacterium Other Than Tuberculosis (MOTTs) ("Tuberculosis")

Mycobacterium avium complex (MAC) and *Mycobacterium intracellulare* are so indistinguishable, that they have been renamed Mycobacterium avium-intracellulare **(MAI)**, which has replaced the vernacular "Mack" for the disseminated disease acquired by AIDS patients. MAI ("Em Ay Eye") does nothing to an immunocompetent host. In AIDS patients (or others with profound immunocompromise, such as transplant patients), MAI presents as **disseminated painless lymphadenopathy** or as a **smoldering pneumonia** without consolidation. These may take weeks to grow positive on culture. They are slow-growing, sensitive to antibiotics, but take a long time to cure. Treatment is with a macrolide (usually azithromycin) and ethambutol. **AIDS CD < 50** requires **weekly prophylaxis** with **azithromycin**.

Mycobacterium marinum causes **granulomas** on the palms of **tropical fish** enthusiasts. It is **noncontagious** and can be treated with isoniazid, rifampin, and ethambutol. *Marinum* for marine, marine for fish and fish tanks.

Mycobacterium ulcerans causes an **ulcer** in Africa. It can be treated with rifampin and streptomycin. *Ulcerans* for ulcers.

BUG	DISEASE	ASSOCIATIONS
Mycobacterium tuberculosis	Primary infection	Macrophages phagocytose TB, die, form granulomas Granulomas, Th1 response, epithelioid macrophages, giant cells Caseating granulomas, few bacteria Ghon complexes are lymph nodes reacting to TB Middle and lower lobes
	Secondary infection	Fever, night sweats, hemoptysis, cavitary lesions PPD + is exposed, X-ray + is latent, Sputum + is active PPD is INH, latent is INH, active is RIPE; B_6 always added Upper lobes
	Miliary TB	Pott's disease = brain Dissemination to all organs, fatal in AIDS BCG vaccine used to prevent miliary TB in children
Mycobacterium leprae	Tubercular leprosy	Well-circumscribed patches, few in number, total anesthesia Th1 response (like tuberculosis, so granulomas) Nerve enlargement, granulomas contain bacteria, few organisms Positive lepromin (leprosy PPD), not contagious
	Lepromatous leprosy	Poorly circumscribed patches, large in number, patchy anesthesia Th2 immune response (unlike tuberculosis, so no granulomas) No nerve enlargement, bacteria on biopsy, limiting granulomas Negative lepromin, very contagious
Mycobacterium avium-intracellulare Complex	MAC	AIDS, CD4 < 50 gets azithromycin prophylaxis Does not cause disease in immunocompetent Disseminated painless lymphadenopathy in immunocompromised
Mycobacterium marinum	Fish tank granulomas	Fish tanks, fish, finger
Mycobacterium ulcerans	Africa ulcers	Little to know

Table 14.2: Mycobacterial Summary